

Supporting Information

DNA-mediated oxidation of p53

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Supplimental Figure S1:

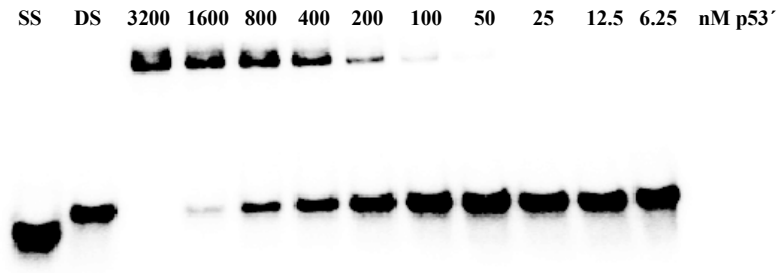


Figure S1: Representative autoradiogram of the EMSA assay to determine binding affinity of p53' to synthetic consensus sequence oligonucleotides. The AQ-AAA sequence is used in the EMSA above. The left two lanes are single stranded oligo (SS) and double stranded oligo (DS), respectively. The following lanes are a serial dilution of p53' starting ranging from 3.2 μ M to 6.25 nM. Samples contained 100 nM AQ-AAA, 5 μ M dAdT, 0.1% NP-40, 0.1 mg/ml BSA in 20 mM TrisCl (pH 8), 20% glycerol, 100 mM KCl, and 0.2 mM EDTA. Image quant was used to analyze the signal intensities for each band. A minimum of three replicates per sequence was conducted. The data were fit to the Hill equation in Origin and the concentration of p53' (in monomer units) at which the DNA is half maximally bound was determined.

Supplemental Figure S2:

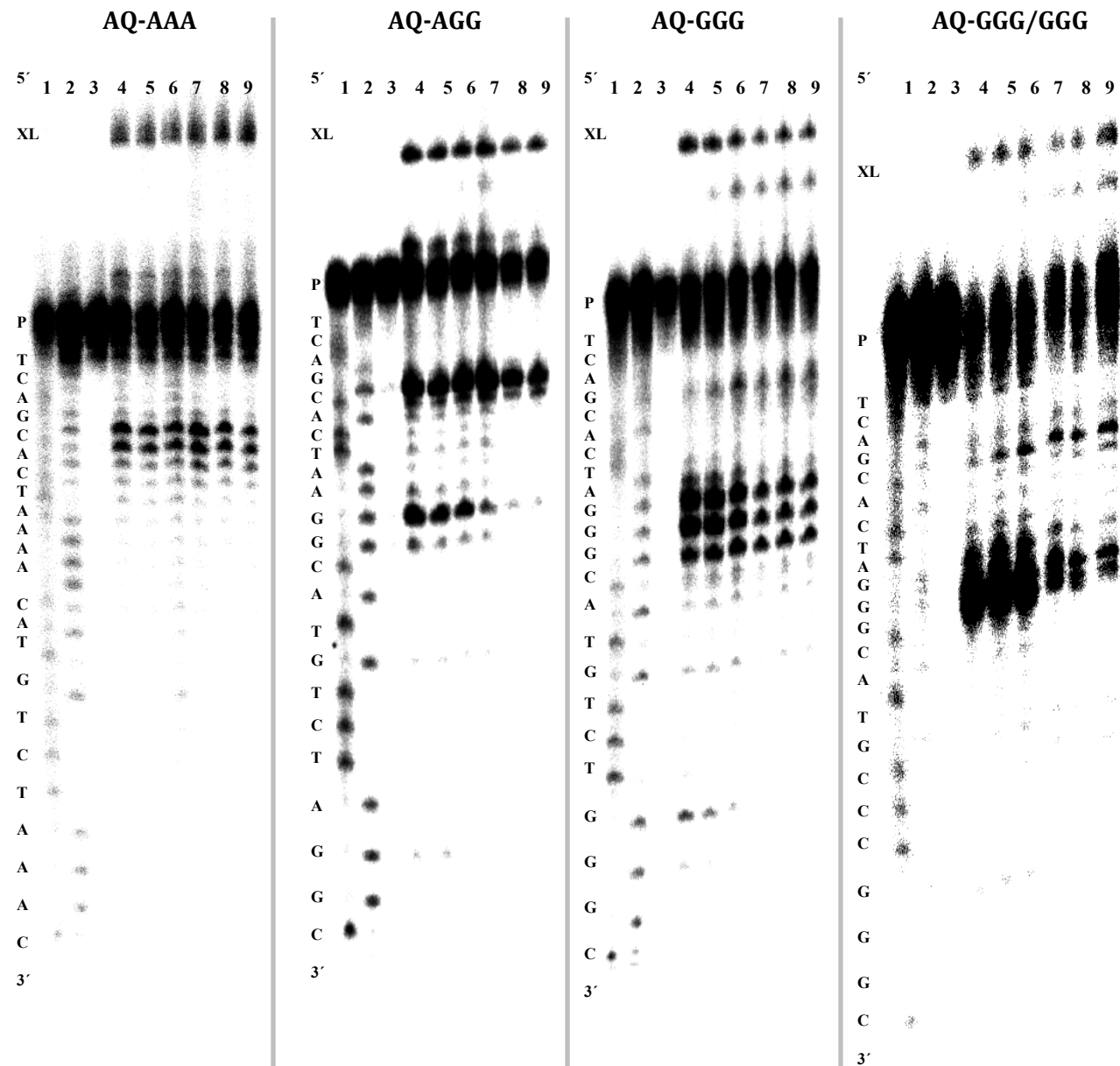


Figure S2: Representative autoradiograms of the guanine oxidation gel shift assays of the AQ-conjugated synthetic consensus sequence oligonucleotides with a 3' radiolabel. Lanes 1 and 2 are the Maxam-Gilbert sequencing lanes corresponding to pyrimidies (C/T) and purines (A/G). Individual bases are designated on the left along with the parent band (P) and the crosslinked bands (XL). The dark control in lane 3 was not irradiated and contained no p53'. The following lanes 4-9 are irradiated samples with varied concentrations of p53' from 0 to 40 μM of p53' monomer respectively. Samples contained 1 μM AQ-Duplex, 5 μM dAdT, 0.1% NP-40, 0.1 mg/ml BSA in 20 mM TrisCl (pH 8), 20% glycerol, 100 mM KCl, and 0.2 mM EDTA. Ethanol precipitated samples were suspended in formamide loading dye and run on a pre-run 20% polyacrylamide denaturing gel at 90 watts for three hours in 1x TBE buffer.

Supporting Table S1:
Predicted responsiveness to DNA CT of select human p53 response elements

Gene	First quarter site	Second quarter site	Linker	Third quarter site	Fourth quarter site	Predicted Responsiveness	p53 Activity	Predicted CT Response of p53	Δ gene regulation	Reference
AIFM2	AGGCA	TGAGC	CACCGTGCCT	GGCCA	AGCCC	AIFM2	Activator	Dissociation	Downregulation	1
	Yes	Yes	Traps	Yes	Yes	Yes				
APAF1	AGACA	TGTCT	GGAGACCCTAGGA	CGACA	AGCCC	APAF1	Activator	Dissociation	Downregulation	2
	No	No	Traps	No	Yes	No				
BBC3	CTGCA	AGTCC		TGACT	TGTCC	BBC3	Activator	Dissociation	Downregulation	3
	Yes	Yes		No	Yes	Yes				
C12orf5	AGACA	TGTCC	AC	AGACT	TGTCT	C12orf5	Activator	Dissociation	Downregulation	4
	No	Yes		No	No	No				
CCNK	AAACT	AGCTT	GC	AGACA	TGCTG	CCNK	Activator	Dissociation	Downregulation	5
	No	Yes		No	Yes	Yes				
CDKN1A	GAACA	TGTCC		CAACA	TGTTG	CDKN1A	Activator	Remains Bound	No Change	6
	No	Yes		No	No	No				
DDB2	GAACA	AGCCC	T	GGGCAT	TGTTT	DDB2	Activator	Dissociation	Downregulation	7
	No	Yes		Yes	No	Yes				
FAS	GGACA	AGCCC		TGACA	AGCCA	FAS	Activator	Dissociation	Downregulation	8
	Yes	Yes		No	Yes	Yes				
GADD45A	GAACA	TGTCT		AAGCAT	TGCTG	GADD45A	Activator	Dissociation	Downregulation	9
	No	No		Yes	Yes	Yes				
IGFBP3	AAACA	AGCCA	C	CAACA	TGCTT	IGFBP3	Repressor	Dissociation	Upregulation	10
	No	Yes		No	Yes	Yes				
MMP2	AGACA	AGCCT		GAACT	TGTCT	MMP2	Activator	Dissociation	Downregulation	11
	No	Yes		No	No	No				
PERP	AGGCA	AGCTC		CAGCT	TGTTC	PERP	Activator	Dissociation	Downregulation	12
	Yes	Yes		Yes	No	Yes				
PLK2	AAACA	TGCCT		GGACT	TGCCC	PLK2	Activator	Dissociation	Downregulation	13
	No	Yes		Yes	Yes	Yes				

PPM1J	GAACA No	TGCCT Yes		GAGCA Yes	AGCCC Yes	PPM1J Yes	Activator	Dissociation	Downregulation	14
PTEN	GAGCA Yes	AGCCC Yes (No)	CAGGCAGCTACACT Traps	GGGCA Yes	TGCTC Yes	PTEN Yes	Activator	Dissociation	Downregulation	15
RRM2B	TGACA No	TGCCC Yes		AGGCA Yes	TGTCT No	RRM2B Yes	Activator	Dissociation	Downregulation	16
SCARA3	GGGCA Yes	AGCCC Yes		AGACA No	AGTTG No	SCARA3 Yes	Activator	Dissociation	Downregulation	17
TP63	TAACT No	TGTTA No	TTG	AAACA No	TGCTC Yes	TP63 No	Activator	Remain Bound	No Change	18
TSC2	TAACA No	AGCTC Yes	G Trap	GGGCT (No) Yes	AGCCC Yes	TSC2 Yes	Activator	Dissociation	Downregulation	19
VCAN	AGACT No	TGCCA Yes	C	AGACA No	AGTCC Yes	VCAN Yes	Activator	Dissociation	Downregulation	20
VDR	TAACT No	AGTTT No		GAACA No	AGTTG No	VDR No	Activator	Remains Bound	No Change	21

1. Wu, M., Xu, L. G., Su, T., Tian, Y., Zhai, Z., and Shu, H. B. (2004) AMID is a p53-inducible gene downregulated in tumors, *Oncogene* 23, 6815–6819.
2. Robles, A., Bemmels, N., Foraker, A., and Harris, C. (2001) APAF-1 is a transcriptional target of p53 in DNA damage-induced apoptosis, *Cancer Res.* 61, 6660-6664.
3. Nakano, K. and Vousden, K. H. (2001) PUMA, a novel proapoptotic gene, is induced by p53, *Mol. Cell* 7, 683–694.
4. Bensaad, K., Tsuruta, A., Selak, M., Vidal, M., Nakano, K., Bartrons, R., Gottlieb, E., and Vousden, K. (2006) TIGAR, a p53-inducible regulator of glycolysis and apoptosis, *Cell* 126, 107-120.
5. Mori, T., Anazawa, Y., Matsui, K., Fukuda, S., Nakamura, Y., and Arakawa, H. (2002) Cyclin K as a Direct Transcriptional Target of the p53 Tumor Suppressor, *Neoplasia* 4, 268–274.
6. Saramäki, A., Banwell, C., Campbell, M., and Carlberg, C. (2006) Regulation of the human p21(waf1/cip1) gene promoter via multiple binding sites for p53 and the vitamin D3 receptor, *Nucleic Acids Res.* 34, 543-554.
7. Tan, T. and Chu, G. (2002) p53 binds and activates the xeroderma pigmentosum DDB2 gene in humans but not mice, *Mol. Cell. Biol.* 22, 3247-3254.
8. Müller, M., Wilder, S., Bannasch, D., Israeli, D., Lehlbach, K., Li-Weber, M., Friedman, S., Galle, P., Stremmel, W., Oren, M., and Krammer, P. (1998) p53 activates the CD95 (APO-1/Fas) gene in response to DNA damage by anticancer drugs, *J. Exp. Med.* 188, 2033-2045.
9. Tamura, R., de Vasconcellos, J., Sarkar, D., Libermann, T., Fisher, P., and Zerbini, L. (2012) GADD45 proteins: Central players in tumorigenesis, *Curr. Mol. Med.* 12, 634-651.
10. Buckbinder, L., Talbott, R., Velasco-Miguel, S., Takenaka, I., Faha, B., Seizinger, B. R., and Kley, N. (1995) Induction of the growth inhibitor IGF-binding protein 3 by p53, *Nature* 377, 646-649.

11. Bian, J. and Sun, Y. (1997) Transcriptional activation by p53 of the human type IV collagenase (gelatinase A or matrix metalloproteinase 2) promoter, *Mol. Cell. Biol.* 17, 6330-6338.
12. Reczek, E. E., Flores, E. R., Tsay, A. S., Attardi, L. D., and Jacks, T. (2003) Multiple response elements and differential p53 binding control Perp expression during apoptosis. *Mol. Cancer Res.* 1, 1048-1057.
13. Burns, T. F. (2003) Silencing of the novel p53 target gene Snk/Plk2 leads to mitotic catastrophe in paclitaxel (taxol)-exposed cells, *Mol. Cell. Biol.* 23, 5556-5571.
14. Shiio, Y., Yamamoto, T., and Yamaguchi, N. (1992) Negative regulation of Rb expression by the p53 gene product, *Proc. Natl. Acad. Sci. U. S. A.* 89, 5206-5210.
15. Stambolic, V., MacPherson, D., Sas, D., Lin, Y., Snow, B., Jang, Y., Benchimol, S., and Mak, T. W. (2001) Regulation of PTEN transcription by p53, *Mol. Cell* 8, 317–325.
16. Kuo, M.-L., Sy, A., Xue, L., Chi, M., Lee, M., Yen, T., Chiang, M.-I., Chang, L., Chu, P., and Yen, Y. (2012) RRM2B suppresses activation of the oxidative stress pathway and is up-regulated by p53 during senescence, *Sci. Rep.* 2, 822.
17. Herzer, K., Falk, C. S., Encke, J., Eichhorst, S. T., Ulsenheimer, A., Seliger, B., and Krammer, P. H. (2003) Upregulation of major histocompatibility complex class I on liver cells by hepatitis C virus core protein via p53 and TAP1 impairs natural killer cell cytotoxicity, *J. Virol.* 77, 8299–8309.
18. Harmes, D. C., Bresnick, E., Lubin, E. A., Watson, J. K., Heim, K. E., Curtin, J. C., Suskind, A. M., Lamb, J., and DiRenzo, J. (2003) Positive and negative regulation of deltaN-p63 promoter activity by p53 and deltaN-p63-alpha contributes to differential regulation of p53 target genes, *Oncogene* 22, 7607–7616.
19. Feng, Z., Hu, w., de Stanchina, E., Teresky, A. K., Jin, S., Lowe, S., and Levine A. J. (2007) The regulation of AMPK beta1, TSC2, and PTEN expression by p53: stress, cell and tissue specificity, and the role of these gene products in modulating the IGF-1-AKT-mTOR pathways, *Cancer Res.* 67, 3043–3053.
20. Yoon, H., Liyanarachchi, S., Wright, F. A., Davuluri, R., Lockman, J. C., de la Chapelle, A., and Pellegata, N. S. (2002) Gene expression profiling of isogenic cells with different TP53 gene dosage reveals numerous genes that are affected by TP53 dosage and identifies CSPG2 as a direct target of p53, *Proc. Natl. Acad. Sci. U. S. A.* 99, 15632-15637.
21. Maruyama, R., Aoki, F., Toyota, M., Sasaki, Y., Akashi, H., Mita, H., Suzuki, H., Akino, K., Ohe-Toyota, M., Maruyama, Y., Tatsumi, H., Imai, K., Shinomura, Y., and Tokino, T. (2006) Comparative genome analysis identifies the vitamin D receptor gene as a direct target of p53-mediated transcriptional activation, *Cancer Res.* 66, 4574-4583.